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Variation of biochemical and antioxidant activity with respect to the part of *Capsicum annuum* fruit from Tunisian autochthonous cultivars

Karima Lahbib[a], Samia Dabbou[b,c,*], Safia EL Bok[a], Gaetano Pandino[d], Sara Lombardo[d], Mohamed EL Gazzah[a]

[a] Laboratory of Biodiversity, Biotechnology and Climatic Change, Faculty of Sciences of Tunis, University of Tunis El Manar, 2092 El Manar II, Tunis, Tunisia
[b] Laboratory of Bioresources, Integrative Biology and Valorisation, Higher Institute of Biotechnology of Monastir, Av. Tahar Hadded, BP 74, 5000 Monastir, University of Monastir, Tunisia
[c] Dentistry Faculty, Avicenne Street, 5019 Monastir, University of Monastir, Tunisia
[d] Department of Agriculture, Food and Environment, University of Catania, via Valdisavoia 5, 95123 Catania, Italy

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ABSTRACT

Pepper is a good source of bioactive compounds and has an important role in the Mediterranean diet as a health-promoting food. Thus, this study was carried out with the aim to evaluate differences among fruit parts (pericarp, placenta and seed) in terms of total phenols and flavonoids content, capsaicin content and antioxidant activity in eleven cultivars grown in Tunisia. The antioxidant activity of the samples was measured by both 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferrous reducing antioxidant power (FRAP) assays. Overall, significant differences were observed in relation to the cultivar and fruit parts. In particular, the pericarp exhibited the highest levels of total phenols (6.82 ± 0.39 mg GAEg^{-1} DW) and flavonoids (0.46 ± 0.03 mg NAEg^{-1}), whereas placenta showed the highest content of capsaicin (0.83 ± 0.07 mg^{-1} DW). Total phenols and flavonoids appeared to be the main contributors to the antioxidant activity in the pericarp, while capsaicin mainly contributed to the antioxidant capacity in the placenta. The fruit parts of Tunisian pepper cultivars demonstrated a high diversity of bioactive compounds, that should be exploited for food and pharmaceutical applications, meantime our data also encouraged the consumption of placenta and seeds. Finally, such wide variability among Tunisian pepper cultivars might represent a good tool for further breeding programs.

1. Introduction

The *Capsicum* genus belongs to the Solanaceae family and includes peppers with important economic value (Giuffrida et al., 2013). They are very popular spices in various parts of the world, mainly due to their attributes of color, pungency and aroma (Bogusz Junior et al., 2015; Sousa et al., 2006). In Tunisia peppers are one of the most popular fruits, grown throughout the country, and show a wide variation in relation to the size, flavor and pungency. The main domesticated species of *Capsicum* peppers is *Capsicum annuum* L., which are known to be consumed fresh, dried, preserved or in spicy sauces as well as coloring and flavor agents in different types of foods (Bogusz Junior et al., 2015; Pino et al., 2007). Their color vary from green, yellow, orange, red, to black (Long-Solís, 1998). Green peppers are harvested before reaching the full maturity and have a slightly bitter flavor than red peppers. Yellow and orange peppers are more mature than green ones, while red peppers are collected at full maturity and have a sweet taste (Mitic et al., 2013). In general, the consumers prefer to consume the pericarp of the fruit and discard placenta and seeds.

Pepper fruits are an excellent source of secondary metabolites, including vitamin C, carotenoids, capsicinoids and flavonoids (Korkutata and Kavaz, 2015; Wahyuni et al., 2013; Zhuang et al., 2012). Being in plant of pepper, these natural products were used in traditional medicine as a source of treatment of primary healthcare. With increase of medicinal plants usage, metabolites and bioactive components are being probed and targeted as novel pharmaceutical leads (Zengin et al., 2017).

Peppers are the only plant genus able to produce capsaicinoids, which are responsible for their typical pungency. Capsaicin is the most predominant and naturally occurring alkamide found in *Capsicum* fruits, reaching around 90% of total capsaicinoids with dihydrocapsaicin (Barbero et al., 2008; De Aguiar et al., 2013). The potential applications of capsaicin range from food flavorings to therapeutics. Previous records suggest pleiotropic pharmacological activities of capsaicin such as an analgesic, anti-obesity, anti-pruritic, anti-inflammatory, anti-apoptotic, anti-cancer, anti-oxidant, and neuro-protective

* Corresponding author at: Dentistry Faculty, Avicenne Street, 5019 Monastir, University of Monastir, Tunisia.
E-mail address: dabbou_samia@yahoo.fr (S. Dabbou).

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functions (Basith et al., 2016). Moreover, it shows gastro-protective effects and has clinical significance for treating vascular-related diseases and metabolic syndrome (Luo et al., 2011). The missing of potent drugs for management of such disorders pushes the requirement for further research into the pharmacological aspects of capsaicin.

Furthermore, red pepper is an excellent source of polyphenols, mainly flavonoids (Lee et al., 1995), which have a strong antioxidant activity and anticancer effects by stimulating the immune system, preventing cardiovascular diseases, and delaying the aging process (Chandrasekara and Shahidi, 2011; Chuah et al., 2008).

In this light, the study of phytochemicals profile in peppers becomes very important to satisfy consumer's demand for healthy foods. Previous studies have been focused to determine the total phenols profile and antioxidant activity in the whole fruit or in a singular part by comparison of few cultivars (Howard et al., 2000), while little attention has been taken on the variation of phytochemicals among pepper parts (pericarp, placenta and seed). Considering that phytochemicals content may vary according to plant parts and cultivars, the aim of this study was to evaluate the total phenols and flavonoids content, capsaicin content and antioxidant activity by DPPH and FRAP assays in the different pepper fruit parts of eleven cultivars widely cultivated in Tunisia.

2. Materials and methods

2.1. Plant material

Eleven autochthonous cultivars of pepper ('Baklouti Chébika', 'BaklC'; 'Beldi', 'Bel'; 'Chaabani', 'Chba'; 'Sisseb Chébika', 'SisC';'Bkalti','Bka'; 'Knaiss','Kna'; 'Baklouti Sbikha','BaklS'; 'Sisseb Sbikha', 'SisS'; 'Fort Menzel Temim', 'FkbM';'Fort de Korba' 'FkbK' and 'Corne de Gazelle', 'CGaz') were grown in the experimental field of the Faculty of Sciences of Tunis, University of Tunis, Tunisia (36°50′N, 10°08′E). Seeds were planted in 19.5-L plastic pots filled with peat and sand mixture (3:1), pH 7.0, during late January 2013in a greenhouse. Eight-weeks-old seedlings were transplanted in the experimental field by a randomized complete block design with three replications in individual plots of 3 × 1.2 m, containing fine sandy loam soil type (pH 8.5) and were irrigated two times per week. Fertilizers were incorporated into the soil at the time of planting. Pepper plants were also covered by protection nets against pests. Ten fully developed fruits of each cultivar were harvested per replication. Fruits were manually separated into three parts: pericarp, placenta and seeds. All the samples were dried at 30 °C up to constant weight and then subjected to the following biochemical analyses.

2.2. Capsaicin content

Capsaicin (CAP) content of the pepper was estimated by spectrophotometric measurement of the blue coloured component formed as a result of reduction of phosphomolybdic acid to lower acids of molybdenum (Sadasivam and Manikkam, 1992). Two grams of sample were extracted with 10 mL of acetone using pestle and mortar. After centrifugation at 10,000 rpm for 10 min, 1 mL of supernatant was recuperated and evaporated to dryness in a hot water-bath. The obtained residue was then dissolved in 0.4 mL of NaOH and 3 mL of 3% phosphomolybdic acid. The resulting solution was shaken and allowed to stand for 1 h at room temperature. Then, it was filtered and centrifuged at 5000 rpm for 15 min. The absorbance of the clear blue solution was read at 650 nm against a blank. The concentration of capsaicin in pepper extracts was expressed as mg of capsaicin equivalents g^{-1} of dry weight (DW).

2.3. Preparation of plant extracts for total phenols, total flavonoids and antioxidant activity analysis

To prepare plant extracts, 10 mL of methanol 80% was added to 1 g of dry pepper sample. The extractions using methanol or ethanol as solvents are the most common where degradation of phenolic compounds is usually avoided (Llorent-Martínez et al., 2016). The obtained suspension was stirred in a water bath at 30 °C for 30 min. The suspension was then centrifuged for 10 min at 1500 g, and supernatants were collected at 4 °C before using.

2.4. Total phenols content

Total phenols (TP) content of pepper extracts was determined using the Folin-Ciocalteu colorimetric method as reported by Singleton and Rossi (1965) and Savran et al. (2016). Briefly, 100 µL of each sample was mixed with 100 µL of Folin-Ciocalteu reagent keeping for 3 min at room temperature. Then, 40 µL of sodium carbonate (75 g/L) and 120 µL of distilled water were added. After mixing, the solution was left at room temperature for 20 min and then the absorbance was recorded at 725 nm. Gallic acid was used for calibration of the standard curve. The results were expressed as mg gallic acid equivalent (GAE) g^{-1} DW.

2.5. Total flavonoids content

The content of total flavonoids (TF) was determined by colorimetric assay using $AlCl_3$ method (Mocan et al., 2016; Um and Kim, 2007) with slight modification. An aliquot (100 µL) of extract was mixed with 30 µL of sodium nitrite (5%). After 5 min, 30 µL of aluminum chloride (10%) were added and the mix was left for 6 min at room temperature. Finally, 100 µL of 1 N NaOH and 25 µL of distilled water were added. The absorbance was recorded at 415 nm. The total flavonoids content was expressed as mg naringin equivalent (NAE) g^{-1} DW.

2.6. DPPH scavenging assay

The antioxidant activity was evaluated by DPPH radical scavenging method according to the procedure of Brand-Williams et al. (1995). An aliquot (50 µL) of methanolic extract was added to 950 µL of DPPH radical. The reaction mixture was vortexed and kept at room temperature in darkness. The absorbance of the samples was measured at 515 nm after 30 min. The antioxidant activity was expressed as the percentage of decline of the absorbance, relative to the control, corresponding to the percentage of DPPH that was scavenged. The percentage of scavenged DPPH (%DPPHsc), was calculated using the formula:

$$\%\ \text{DPPH sc} = (\text{Acont} - \text{Asamp}) \times 100/\text{Acont}$$

Where Acont is the absorbance of the control and Asamp the absorbance of the sample.

2.7. Ferric reducing power assay

Antioxidant activity was also measured using FRAP assay (Benzie and Strain, 1996; Simirgiotis et al., 2016). FRAP reagent consisted of 10 $mmolL^{-1}$of 2,4,6-tripyridyl-*S*-triazine (TPTZ) in 40 $mmolL^{-1}$ofHCl, 20 $mmolL^{-1}$ ferric chloride and 300 $mmolL^{-1}$of sodium acetate buffer (pH 3.6) in the ratio of 1:1:10 (v/v/v). The FRAP reagent (3 mL) was combined to the extract (100 mL) and mixed thoroughly. After standing at room temperature for 4 min, the absorbance was measured at 593 nm against blank. The standard curve was performed using Trolox. Results were expressed as Trolox equivalent (TE) in $mmolg^{-1}$ DW.